

TransNGS® Tn5 DNA Library Prep Kit for Illumina® (for 5 ng DNA)

Please read the manual carefully before use

Catalog No. KP121

Storage: at -70°C or below for one year (at -18°C or below for 6 months)

Description

TransNGS® Tn5 DNA Library Prep Kit for Illumina® is a reagent kit designed for Illumina high-throughput sequencing platform, suitable for preparing DNA library from 5 ng initial sample (such as acquiring small genome, plasmid and PCR amplification product larger than 300 bp). The resulting library can be used for single-end or paired-end sequencing. **This kit employs an in vitro transposition technique, which can simultaneously complete DNA fragmentation and adapter ligation within 5 minutes.** In comparison to conventional library preparation method that requires DNA fragmentation, end repair, and adapter ligation, the in vitro transposition technique significantly shortens library construction time, simplifies the operation and requires less DNA input.

Feature

- Time saving and simple operation
- Require small amount of DNA

Application

Prepare short fragment DNA library for Illumina high-throughput sequencing platform from purified small genome (Bacteria, Archaea, dsDNA Virus, etc.), plasmid and PCR amplification product larger than 300 bp.

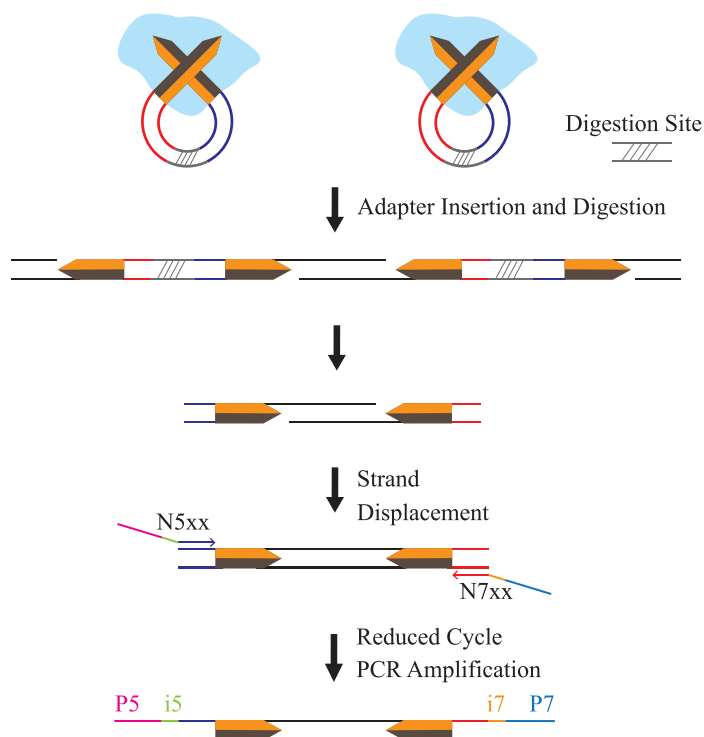
Kit content

| Component | KP121-11 (12 rxns) | KP121-03 (96 rxns) |
|---|--------------------|--------------------|
| Tn5-5 Enzyme Mix | 24 µl | 192 µl |
| 5×Insertion and Digestion Buffer II | 36 µl | 288 µl |
| 4×Stop Buffer | 60 µl | 480 µl |
| TransNGS® Tn5 Library Amplification SuperMix (2×) | 300 µl | 4×600 µl |
| Library Elution Buffer (LEB) | 600 µl | 5 ml |
| Nuclease-free Water | 1 ml | 5 ml |

Initial Sample Requirement

The initial sample should be purified DNA dissolved in Nuclease-free Water or 10 mM Tris-HCl (pH8.0), with an OD_{260}/OD_{280} value between 1.8-2.0. Fluorescent dye method (Qubit or PicoGreen) is chosen for specific dsDNA detection and DNA concentration measurement. The ratio of the concentration measured by absorbance method (such as Nanodrop) to the concentration measured by fluorescent dye method for the same DNA sample is ≤ 2 .





| Component | Volume |
|--|------------|
| Tn5-5 Enzyme Mix | 2 μ l |
| 5 ng DNA | Variable |
| 5 \times Insertion and Digestion Buffer II | 3 μ l |
| Nuclease-free Water | Variable |
| Total volume | 15 μ l |



- (2) Mix by pipetting up and down, if there is liquid on the walls of the tube, spin briefly and immediately proceed to the next step of the reaction.
- (3) Place the reaction tube in a thermocycler and incubate at 55°C for 5 minutes (lid temperature at 70°C).
- (4) Immediately place the reaction tube on ice, and immediately add 5 µl of 4× Stop Buffer to the tube. Mix by pipetting up and down, and place the tube on ice.

2. Library Amplification

- (1) Add the following reaction components to the PCR tube on ice:

| Component | Volume |
|--|--------|
| Output of previous reaction | 20 µl |
| <i>TransNGS</i> [®] Tn5 Library Amplification SuperMix (2×) | 25 µl |
| N5xx* | 2.5 µl |
| N7xx* | 2.5 µl |
| Total volume | 50 µl |

* *TransNGS*[®] Tn5 Index Kit for Illumina[®] (Cat. No. KI101) provides 8 different N5xx barcode oligos and 12 different N7xx barcode oligos. Please choose as needed.

- (2) Mix by pipetting up and down. Spin briefly.
- (3) Recommended PCR amplification program:

| | | |
|-------|--------|----------|
| 72°C | 3 min* | |
| 98°C | 3 min | |
| 98°C | 30 sec | 9 cycles |
| 62°C | 30 sec | |
| 72°C | 30 sec | |
| 72°C | 3 min | |
| ≤10°C | Hold | |

* This step is indispensable. The transposition reaction product is not complete double-stranded DNA. Incubate at 72°C for 3 minutes to generate complete PCR templates.

3. Size selection of library amplification product

If there is no special requirement for fragment length, the enriched library can directly be purified with 1.0× DNA beads, rather than going through size selection steps.

MagicPure[®] Size Selection DNA Beads (Cat. No. EC401) is recommended for size selection of library amplification product. To avoid residual adapter or residual long fragments, it is recommended to perform fragment size selection twice, or to conduct DNA purification with 1.0× magnetic beads before size selection. The specific steps for fragment size selection are as follows:

- (1) Pre-warm the DNA beads for 30 min from 2-8°C to room temperature.
- (2) Transfer 50 µl output from library amplification step into a 1.5 ml sterile centrifuge tube. If the output is less than 50 µl, it should be supplemented with Nuclease-free Water.
- (3) Vortex the magnetic beads to ensure thorough mixing. Add the appropriate volume of DNA beads to the PCR products (refer to table1).

Volume of DNA beads to add = volume of DNA solution × 1st volume ratio.

For example: 30 µl beads = 50 µl DNA solution × 0.6

- (4) Mix the sample thoroughly by pipetting up and down, then let it stand at room temperature for 5 minutes.

Note: Insufficient mixing will significantly affect the experimental results.



- (5) Place the 1.5 ml tube on a Magnetic Stand. Let it stand at room temperature for approximately 5 minutes until the solution becomes clear, allowing the magnetic beads to fully adhere to the tube wall close to the magnetic rack.
Note: If there is liquid on the tube wall, briefly centrifuge and then place it on the magnetic rack. Please make sure that all magnetic beads are fully attached to the tube wall.
- (6) Keep the 1.5 ml tube on the Magnetic Stand, transfer the supernatant into another clean 1.5 ml tube. Discard the beads.
- (7) Add the appropriate volume of beads into the supernatant (refer to Table 1).
Volume of the beads to add = initial volume of DNA solution \times 2nd volume ratio
For example: 7.5 μ l beads = 50 μ l DNA solution \times 0.15
- (8) Mix by pipetting up and down. Incubate at room temperature for 5 minutes.
Note: Insufficient mixing will significantly affect the experimental results.
- (9) Place the 1.5 ml centrifuge tube on the magnetic rack and let it stand at room temperature for approximately 5 minutes until the solution becomes clear, allowing the magnetic beads to fully adhere to the tube wall close to the magnetic rack. Discard the supernatant.
Note: If there is liquid on the tube wall, briefly centrifuge and then place it on the magnetic rack, ensuring all magnetic beads are fully attached to the tube wall. Do not pipette the magnetic beads, as this may affect the final yield.
- (10) Keep the 1.5 ml tube on the Magnetic Stand, add 200 μ l of freshly prepared 80% ethanol into the tube and keep it at room temperature for 30 seconds without mixing. Then discard the supernatant.
Note: 80% ethanol must be freshly prepared, as it may affect the experimental results.
- (11) Repeat Step (10) once.
- (12) Keep the 1.5 ml tube on the Magnetic Stand with the lid open, dry the beads at room temperature.
Note: Do not heat-dry the tube, as it may affect the final yield.
- (13) Remove the 1.5 ml tube from the Magnetic Stand, add 23 μ l Library Elution Buffer. Mix by pipetting up and down or by vortex. Incubate at room temperature for 2 minutes.
- (14) Place the 1.5 ml tube on the Magnetic Stand and wait for 2 minutes until the solution is clear, ensuring that all magnetic beads are fully attached to the tube walls.
Note: If there is liquid on the tube wall, briefly centrifuge and then place it on the magnetic rack. The room temperature stand time can be extended to 5 minutes, ensuring all magnetic beads are fully attached to the tube wall.
- (15) Carefully transfer 20 μ l of the supernatant into a fresh centrifuge tube, and store at -20°C .

Appendix

Table 1 Reference conditions for size selection by *MagicPure*[®] Size Selection DNA Beads

| | | | |
|--|-------|-------|-------|
| Average Size of Selected Library (bp) | ~330 | ~480 | ~680 |
| Insert Size of Selected Library (bp) | ~300 | ~350 | ~550 |
| 1st volume ratio (DNA Beads : DNA) | 0.65× | 0.6× | 0.55× |
| 2nd volume ratio (DNA Beads : DNA) | 0.15× | 0.15× | 0.12× |



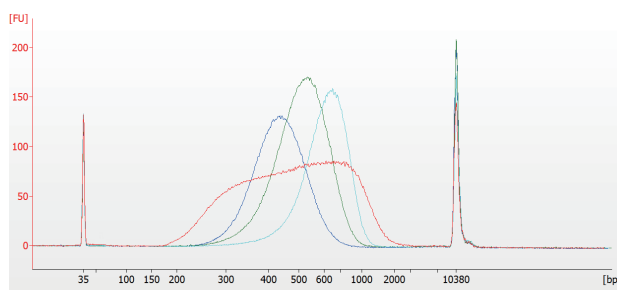


Figure 1 Reference of size selected library by *MagicPure*® Size Selection DNA Beads.
Library amplification output - Bacteria library prepared by this kit, after 9 PCR cycles and 1.0× beads purification; or after 9 PCR cycles and size selection by 0.65+0.15; 0.6×+0.15×; or 0.55×+0.12× beads.

Note

If slight precipitate or turbid liquid is visible after thawing 4×Tn5 Digestion Mix, dissolve at 37°C water bath and mix thoroughly before use.

The transposase recognition sites exhibit a certain preference, resulting in each inserted fragment having 9 nucleotides at both ends that are not entirely randomly distributed, which means the first 9 nucleotides of each sequencing read show a certain preference (Table 2).

Table 2 Transposase recognition site preference statistics table

| Probability Statistics (%) | | | | | | | | | |
|----------------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Base position | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| A | 20.0375 | 21.08 | 24.8825 | 16.1325 | 36.29 | 38.2125 | 30.3475 | 34.4075 | 15.02 |
| T | 10.3075 | 36.355 | 29.0425 | 39.1625 | 35.6925 | 17.7975 | 23.9275 | 21.42 | 21.6425 |
| C | 25.125 | 24.66 | 25.1125 | 31.4525 | 13.8025 | 13.2325 | 19.8475 | 19.0425 | 39.17 |
| G | 44.345 | 17.3975 | 20.96 | 13.21 | 14.2175 | 30.7575 | 25.8775 | 25.13 | 24.165 |

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